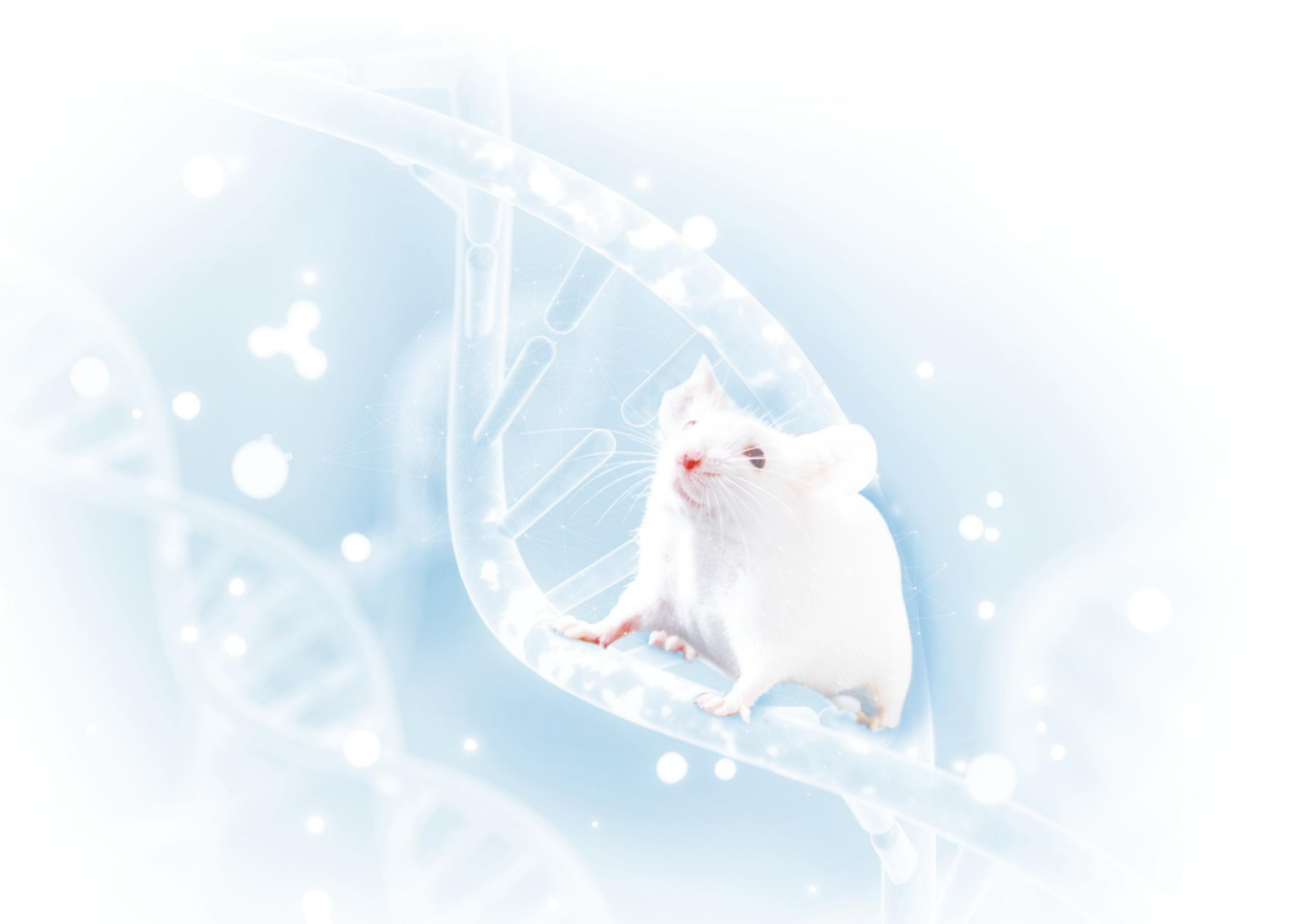


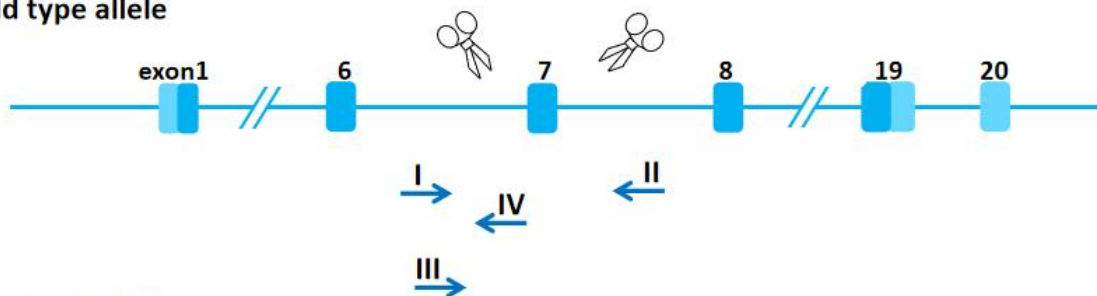
Stxbp1-KO Genotyping Protocol



Common Name	Stxbp1-KO	Cat. NO.	NM-KO-241571
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy

Wild type allele



knockout allele



 : coding region  : uncoding region

 : Cas9/gRNA  : mutation

NHEJ : Non-homologous end joining

 : primer location

Primers

Primer	Sequence (5'→3')	Primer type
P1	GCACACACCACCCCTAAGT	Forward
P2	AGGGGATCTGGCTCACTCAA	Reverse
P3	AGCAAACACCTCACCTCCTT	Forward
P4	AGGCAGGGGTGTAAGTCCT	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder DL2000 bp 10000 8000 6000 5000 4000 3500 3000 2500 2000 1500 1000 750 500 250 1% Tris/Glycine LE G0 Agarose #804971 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min 1.7% agarose
Genotype	Knockout type: -678bp Wild type: P1P2 =1243 bp; P3P4 =434 bp Heterozygote: P1P2 =1243 bp and 565 bp; P3P4 =434 bp Homozygote: P1P2 =565 bp

Note: In both wild-type and heterozygous mice, whether the P1 and P2 primers can amplify larger bands does not affect the interpretation of the results, because the purpose of designing this pair of primers is to amplify KO band

Reaction & Cycling

PCR Reaction System	Reaction Component			Volume (µl)
	ddH ₂ O			8.0
	2×Rapid Taq Master Mix			10.0
	P1(10 pmol/µl) or P3(10 pmol/µl)			0.5
	P2(10 pmol/µl) or P4(10 pmol/µl)			0.5
	Genomic DNA			1.0
	Total			20
	2×Rapid Taq Master Mix from Vazyme(Code Number: P222-01)			
Cycling Reaction	Step	Temp	Time	Note
	1	95°C	5 min	
	2	95°C	20 sec	
	3	60°C	20 sec	
	4	72°C	20 sec	35 repeats to 2
	5	72°C	5 min	
	6	12°C	Hold	